Pergamon

0042-6989(95)00256-1

Vision Res., Vol. 36, No. 11, pp. 1513–1520, 1996
Copyright © 1996 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0042-6989/96 $15.00 + 0.00

Lectin Cytochemical Analysis of Glycoconjugates in Photoreceptor Cell Membranes of *Lampetra japonica*

MAKOTO ISHIKAWA,*§ AKIRA TONOSAKI,† OSAMU HISATOMI,‡ FUMIO TOKUNAGA,‡ TAKESHI KOSEKI,* SHOZO SAKURAGI†

Received 3 July 1995; in revised form 18 September 1995

Seven types of ferritinized lectin were used to examine the distribution of glycoconjugates on the outer segment membranes of lamprey photoreceptor cells. Ultrastructural pre-embedding labeling revealed that peanut agglutinin, soybean agglutinin and *Ricinus communis* agglutinin I were preferentially bound to the proximal, lateral and luminal surfaces of the long cell outer segments, whereas *Griffonia simplicifolia* agglutinin II and concanavalin A agglutinin were bound to the corresponding surfaces of the short cell outer segments. The results indicate that there is marked difference in the composition of glycoconjugates over the outer segment membranes between long and short photoreceptors. Copyright © 1996 Elsevier Science Ltd.

Retina Photoreceptor cell Outer segment membrane Lectin binding *Lampetra japonica* (Cyclostomata)

INTRODUCTION

Glycoconjugates, including glycolipids, glycoproteins and proteoglycans, are present in the disk and plasma membrane of photoreceptor cells. Although the precise function of these molecules is unknown, they may play an important role in phagocytosis by the pigment epithelium (O'Brien, 1976) and the assembly of outer segment membranes (Fliesler & Basinger, 1985). Lectins have been used as probes for the identification and characterization of glycoconjugates on the cell surface (Sharon & Lis, 1972). Through a series of histochemical studies, certain types of lectins have been shown as useful to distinguish between rod and cone photoreceptors in the vertebrate retina (Bridges & Fong, 1980). Concanavalin A (Con A) and wheat germ agglutinin (WGA) bind to mannosyl and *N*-acetyl-glucosaminyl residues constituting the carbohydrate moiety of rhodopsin in the bovine retina (Steinemann & Stryer, 1973; Fukuda *et al.*, 1979). Peanut agglutinin (PNA) is considered to recognize cone cells in the teleostean, amphibian and mammalian retina (Bridges & Fong, 1980; Uehara *et al.*, 1983; Blanks & Johnson, 1984). In this context, the short cell of lamprey retina was identified as a primitive rod, the long cell as a primitive cone (Ishikawa *et al.*, 1989). However, the degree of resolution afforded by prior light microscopic studies has made it difficult to determine whether lectin binding is localized to the actual outer segment membrane in addition to the surrounding interphotoreceptor matrix (Johnson *et al.*, 1986).

In the present study, ultrastructural pre-embedding labeling was undertaken with seven types of ferritin (Fer) conjugated lectins to determine more precisely the distribution of glycoconjugates on the outer segment membranes of the lamprey photoreceptor cells.

MATERIALS AND METHODS

Reagents

The following types of lectin were used in this study: Fer-conjugated PNA, soybean agglutinin (SBA), *Ricinus communis* agglutinin I (RCA), *Griffonia simplicifolia* agglutinin II (GS), Con A, WGA, and *Ulex europaeus* agglutinin I (UEA). The reagents were commercially supplied from E. Y. Laboratories (San Mateo, CA, U.S.A.).

Haptenic sugars, D-galactose (Gal), *N*-acetyl-D-galactosamine (GalNAc), *N*-acetyl-D-glucosamine, methyl-D-mannoside, and L-fucose were used against PNA and RCA, SBA, GS and WGA, Con A, and UEA, respectively.

*Department of Ophthalmology, Akita University School of Medicine, 1-1-1, Hondo, Akita 010, Japan.
†Department of Anatomy, Yamagata University School of Medicine, Japan.
‡Department of Biology, Faculty of Science, Osaka University, Japan.
§To whom all correspondence should be addressed

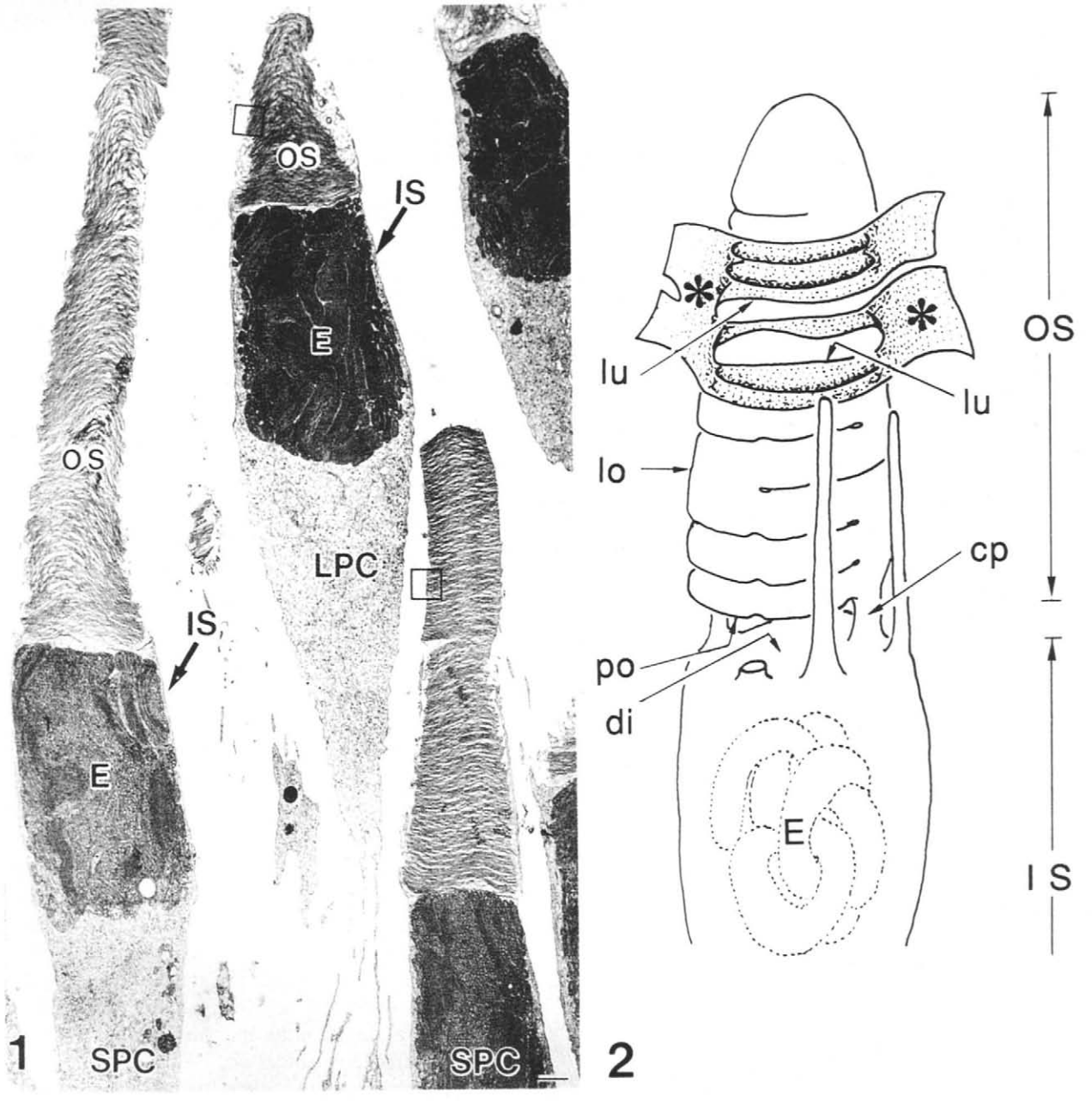

FIGURE 1. Electron micrograph containing long (LPC) and short photoreceptor cells (SPC). The retina is detached from the pigment epithelium, and incubated with Fer-PNA. The lamprey retina is characterized by the conspicuous arrangement of sclerally located LPCs—and vitreally situated SPC—ellipsoid bodies. High magnification views of rectangle areas are enlarged in Fig. 4(a) and (b). Abbreviations: OS, outer segment; IS, inner segment; E, ellipsoid body; bar = 1 μm, $\times$5000.

FIGURE 2. Schematic drawing of long photoreceptor cell of the lamprey retina. A part of the plasma membrane (*) is peeled open and the structures inside the outer segment are exposed. Examined regions consist of proximal (po) and lateral surfaces of the outer segment (lo) as well as the luminal surface (lu) of the disk membrane. The corresponding surfaces of the short photoreceptor cell are also examined. The di and cp represent the distal surface of the inner segment and ciliary plasma membrane, respectively. Position of the ellipsoid body (E) is indicated. Abbreviations: OS, outer segment; IS, inner segment.

Pre-embedding lectin cytochemistry

Adult river lampreys (*Lampetra japonica*) were commercially obtained from local fishery agent. After dark adaptation for 2 hr, the fish were pithed to remove the eyes. The retinas were detached from the pigment epithelium, trimmed into small pieces and fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M) at 4°C for 2 hr. Specimens were washed with phosphate-buffered saline (PBS), followed by incubation with Fer-lectin (1 mg/ml) at 4°C for 4 hr. For the control study, some specimens were incubated with 1 mg/ml Fer-lectin and its haptenic sugar (0.1 M).

To expose the luminal surface of disk membranes and put them in contact with lectins, some unfixed specimens were incubated with hypotonic buffer solution (0.01 M phosphate buffer; pH 7.4) for 5 sec. After fixation with glutaraldehyde, they were frozen in liquid nitrogen slush and thawed out in the Fer conjugated lectin solution at

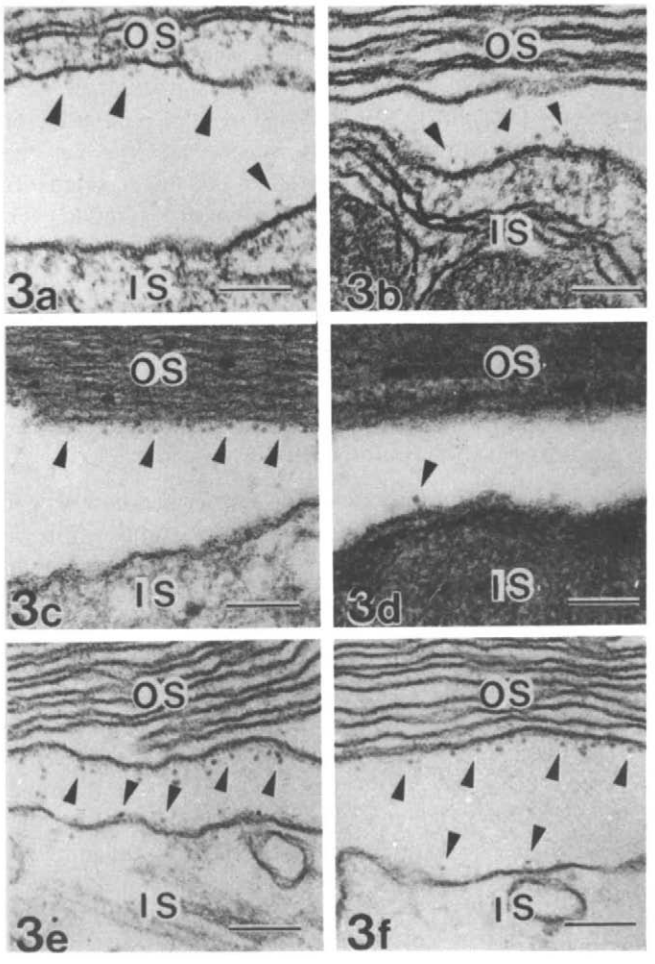

FIGURE 3. Proximal surface of the outer segment (OS) and opposing distal surface of the inner segment (IS) of long (a) and short cell (b) after incubation with Fer-PNA. Note a number of ferritin particles (arrowheads) on the proximal surface of the outer segment, in contrast to the scarcity on the opposing distal surface of the inner segment in long cell. In short cell, such polarity of PNA labeling seems to be reversed. Opposing surfaces of the outer and inner segments of long (c) and short cell (d) after incubation with Fer-SBA. Note a number of ferritin particles (arrowheads) on the proximal surface of the outer segment, in contrast to the absence on the opposing distal surface of the inner segment in long cell. Each surface of the short cell shows vestigial labeling. Opposing surfaces of the outer and inner segments of long (e) and short cell (f) after incubation with Fer-RCA. There seems to be a preference of labeling for the proximal surface of the outer segment, in contrast to less intense labeling on the opposing distal surface of the inner segment in long cell. Such a labeling pattern is reproduced in short cell. Bars = 0.1 μm, ×90,000.

4°C for 4 hr. This procedure was a modification of that previously described by Röhlich (1976). After washing again with PBS, specimens were post-fixed with 1% OsO_4 in 0.1 M phosphate buffer at room temperature for 60 min, dehydrated through a graded ethanol series and embedded in Epon. Ultrathin sections were photographed with the H-700 electron microscope at 30,000 and printed at 90,000 magnification.

RESULTS

The lamprey retina displayed a two-layered arrangement of long and short cells; the ellipsoid bodies of the outer segments of the long cells were located sclerally, whereas those of short cells were situated vitreally (Fig. 1).

TABLE 1. Lectin binding to each surface of the lamprey photoreceptor outer segment membranes

	Proximal surface		Lateral surface		Luminal surface	
	LPC	SPC	LPC	SPC	LPC	SPC
PNA	++	−	++	−	++	−
SBA	++	−	++	−	++	−
RCA	++	++	++	+	++	+
GS	−	++	−	++	−	++
Con A	++	+++	++	+++	++	+++
WGA	++	++	++	+	++	++
UEA	−	−	−	−	−	−

Abbreviations: PNA, peanut agglutinin; SBA, soybean agglutinin; RCA, *Ricinus communis* agglutinin I; GS, *Griffonia simplicifolia* agglutinin II; Con A, concanavalin A agglutinin; WGA, wheat germ agglutinin; UEA, *Ulex europaeus* agglutinin I.
The intensity of lectin labeling was qualitatively evaluated by the density of Fer particles on each surface of outer segments of the long photoreceptor cell (LPC) and short photoreceptor cell (SPC) as negative (−), weak (+), moderate (++), or intense reaction (+++).

In the present ultrastructural labeling, photoreceptor plasma membranes were impermeable to Fer-lectins, so that lectin-binding was limited to the free surfaces of the photoreceptor outer segments. The proximal, lateral and exposed luminal surfaces of the photoreceptor outer segments seemed to be freely accessible to the incubation medium containing Fer-lectins. Therefore, we examined the lectin-binding sites on these three surfaces marked in Fig. 2. The number of the examined regions was 13–15 for each surface of the outer segments taken from different samples. Lectin binding sites were made visible by a variable series of 10 nm Fer particles along the membranes on a electron micrograph. Observations of lectin binding on each surface were summarized for long and short cells in Table 1.

Specific lectin binding for long cell outer segments

On the proximal surfaces of the outer segments, Fer-PNA [Fig. 3(a)] and Fer-SBA [Fig. 3(c)] showed specific labeling for the long cell. On short cell outer segments, by contrast, they attached vestigially to the corresponding surfaces of the short cell outer segments [Figs 3(b) and 3(d)]. There seemed to be no difference in the labeling density of Fer-RCA on the proximal surface of the outer segments between long [Fig. 3(e)] and short cell [Fig. 3(f)].

On the lateral and luminal surfaces of the outer segments, Fer-PNA [Figs 4(a) and 5(a)] and Fer-SBA [Figs 4(c) and 5(c)] showed specific labeling for the long cell. Short cell outer segments were negative for their binding [Figs 4(b), 5(b), 4(d) and 5(d)]. The Fer-RCA was preferentially bound to the lateral [Fig. 4(e)] and luminal surface regions of the long cell outer segments [Fig. 5(e)]. Less intense labeling was obtained on the

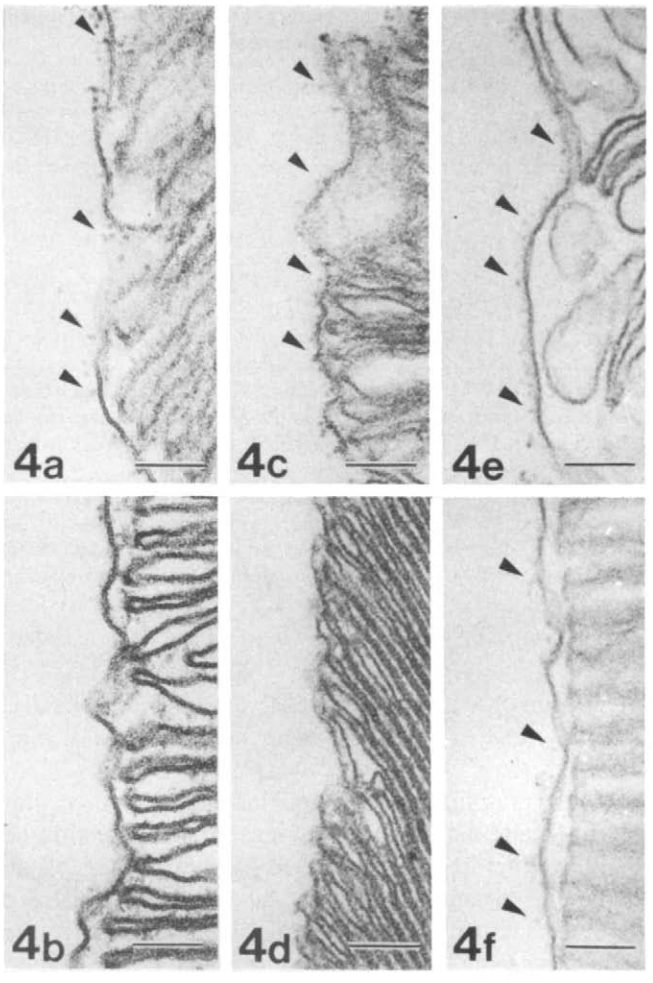

FIGURE 4. Lateral surfaces of the outer segments of long (a) and short cell (b) after incubation with Fer-PNA. A number of Fer-PNA particles (arrowheads) are deposited on the lateral surface of the long cell outer segment, but only a trace of ferritin particles is recognized on the corresponding surface of the short cell outer segment. Lateral surfaces of the outer segments of long (c) and short cell (d) after incubation with Fer-SBA. Note a number of ferritin particles (arrowheads) on the lateral surface of the long cell outer segment, in contrast to the absence on the corresponding surface of the short cell outer segment. Lateral surfaces of the outer segments of long (e) and short cell (f) after incubation with Fer-RCA. Note more numerous ferritin particles (arrowheads) on the lateral surface of the long cell outer segment than those on the corresponding surface of the short cell outer segment. Bar = 0.1 μm, ×90,000.

corresponding surfaces of the short cell outer segments [Fig. 4(f) and 5(f)].

Specific lectin binding for short cell outer segments

On the proximal, lateral and luminal surfaces of the outer segments, Fer-GS showed specific labeling for the short cell [Figs 7(b), 6(a), 6(b) and 9(b)]; the corresponding surfaces of the long cell outer segments were negative [Figs 7(a), 6(b) and 9(a)]. The Fer-Con A showed labeling preference for each surface region of the short cell outer segments [Figs 7(d), 8(b) and 9(d)] compared with the corresponding region of the long cell outer segments [Figs 7(c), 8(a) and 9(c)]. There seemed to be no difference in the labeling density of Fer-WGA on the proximal and luminal surface of the outer segments between long [Figs 7(e) and 9(e)] and short cells [Figs 7(f) and 9(f)]. On the lateral surface of the long cell outer segments [Fig. 8(c)], Fer-WGA showed a preference of labeling, in contrast to less intense labeling on the corresponding surface of the short cell outer segments [Fig. 8(d)]. The Fer-UEA was negative in binding to each surface of the outer segments of both photoreceptor cells [Figs 7(g), 7(h), 8(e), 8(f), 9(g) and 9(h)].

In the specimens incubated with a mixture of Fer-lectin and a specific haptenic sugar, reaction for lectins was remarkably reduced all over the regions.

DISCUSSION

The electron microscopic studies described here reveal that there is marked difference in the composition of glycoconjugates over the outer segment membranes between long and short photoreceptors. The PNA-, SBA- and RCA-binding glycoconjugates were preferentially localized in the photoreceptive membranes of the long cell, whereas GS- and Con A-binding glycoconjugates were localized in those membranes of the short cell.

The PNA, which recognizes Gal-GalNAc disaccharide linkages (Lotan *et al.*, 1975), is known to bind to the cone photoreceptor plasma membrane in addition to the surrounding extracellular matrix sheaths in the monkey retina (Sameshima *et al.*, 1987; Blanks *et al.*, 1988). The present study was initiated to assess whether PNA-binding is associated directly with the disk or plasma membrane, or the surrounding interphotoreceptor matrix (IPM) in the lamprey retina. However, the PNA-positive IPM has not been found around the long cell and Fer-PNA directly binds to the disk or plasma membranes of the long cell outer segments. Our preliminary PNA blotting against the extract of lamprey outer segment membranes developed two obvious bands at 78 and 110 kDa (unpublished data), which were different from those reported in mammalian and avian retinas (Hageman & Johnson, 1986). These results may suggest that there is a difference in the molecular composition of PNA-positive glycoconjugates of lamprey and other higher vertebrates.

In the present study, the binding of SBA, which recognizes GalNAc (Lis *et al.*, 1970), is identical to PNA-binding. The SBA has been reported to bind specifically to the cone outer segment in the frog retina (Nir & Hall, 1979). The SBA, on the other hand, has not shown any binding in the mouse (Blanks & Johnson, 1983) and human retina (Kivelä & Tarkkanen, 1987; Sönderström, 1988). Considering the specificity of SBA for the long cell outer segment, SBA may have a binding preference for cone cells of lower vertebrates rather than those of higher vertebrates.

Although the binding of RCA, which recognizes Gal (Nicolson *et al.*, 1974), is similar to that of PNA in the lamprey photoreceptor, it is not identical. The major difference is that PNA or SBA binds long cell outer segment exclusively, while RCA binds short cell to some extent in addition to long cell. Therefore, RCA cannot be

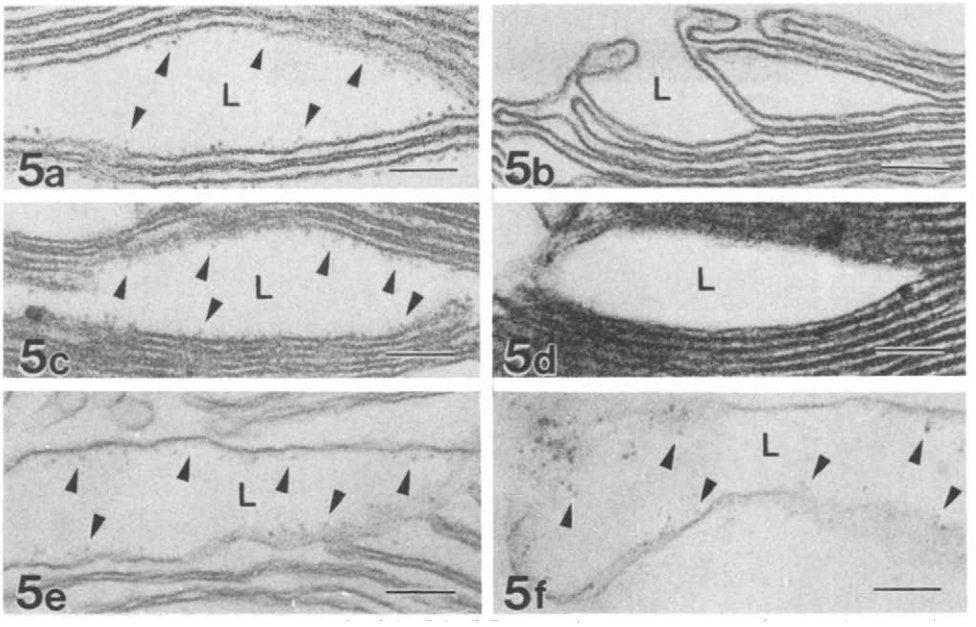

FIGURE 5. Freeze-thawed membranous disks of the long (a) and short cell outer segments (b) after incubation with Fer-PNA. Note a number of ferritin particles (arrowheads) confined to long cell disks, in contrast to their absence in short cell disks. L, luminal cavity. Freeze-thawed membranous disks of the long (c) and short cell outer segments (d) after incubation with Fer-SBA. Note a number of Fer-SBA particles (arrowheads) restricted in long cell disks, in contrast to the absence in short cell disks. Freeze-thawed membranous disks of the long (e) and short cell outer segments (f) after incubation with Fer-RCA. Note more numerous ferritin particles (arrowheads) in long cell disks than those in short cell disks. Bars = 0.1 μm, ×90,000.

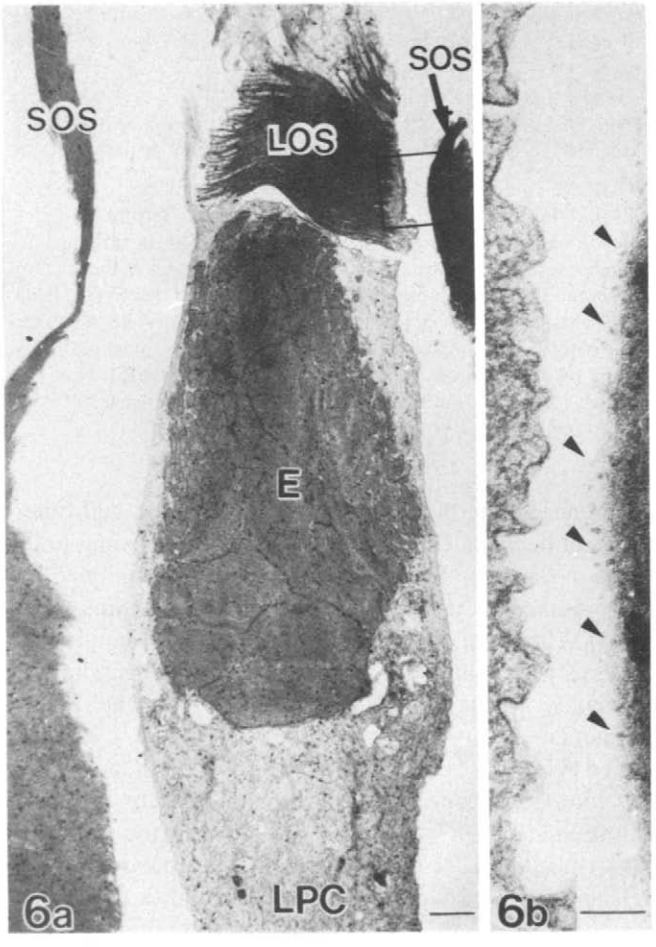

FIGURE 6(a). Electron micrograph of the long (LOS) and short cell outer segments (SOS) after Fer-GS incubation. High magnification view of rectangle area is enlarged in (b). E, ellipsoid body; bar = 1 μm, ×6000. (b) Note a number of ferritin particles (arrowheads) on the lateral surface of the outer segment of short cell, in contrast to the absence on the corresponding surface of the long cell. Bar = 0.1 μm, ×90,000.

considered as a specific probe for long cell-like PNA or SBA. It is of interest that RCA has been reported to preferentially bind to the proximal surface of rod outer segments in the bovine retina (Hicks & Molday, 1985), or to the corresponding surface of cone outer segments in the frog retina (Nir & Hall, 1979). It has been discovered that the proximal portion of the outer segments is stacked with newly synthesized disks (Olive, 1980). Therefore, RCA labeling may suggest that new disks are characterized by Gal-positive glycoconjugates. In the present study, RCA does not show the specific labeling of the proximal surface of the long or short cell outer segments and its binding glycoconjugates seemed to be evenly distributed on each surface of the outer segments of both cells. Since the glycoconjugates may play an important role in the incorporation of visual pigments into disk membranes (Fliesler & Basinger, 1985), the data suggest that lamprey photoreceptor cell may organize and maintain the photoreceptive membranes in a different manner to higher vertebrates.

The GS (isolectin II of *Griffonia simplicifolia*), which recognizes GlcNAc (Lyer *et al.*, 1976), was specifically bound to the short cell outer segment membrane. In the human retina, specific GS-binding to rod photoreceptor outer segments has been reported (Bishop *et al.*, 1993). The result is particularly interesting, as it suggests that GS can be used as a specific probe for the photoreceptive membrane of rod-type cell in the vertebrate retina.

Con A and WGA have been considered to bind to bovine rhodopsin molecule (Steinemann & Stryer, 1973; Fukuda *et al.*, 1979). Therefore, Con A and WGA are expected to show identical labeling distribution on rod outer segments. However, the present study revealed a

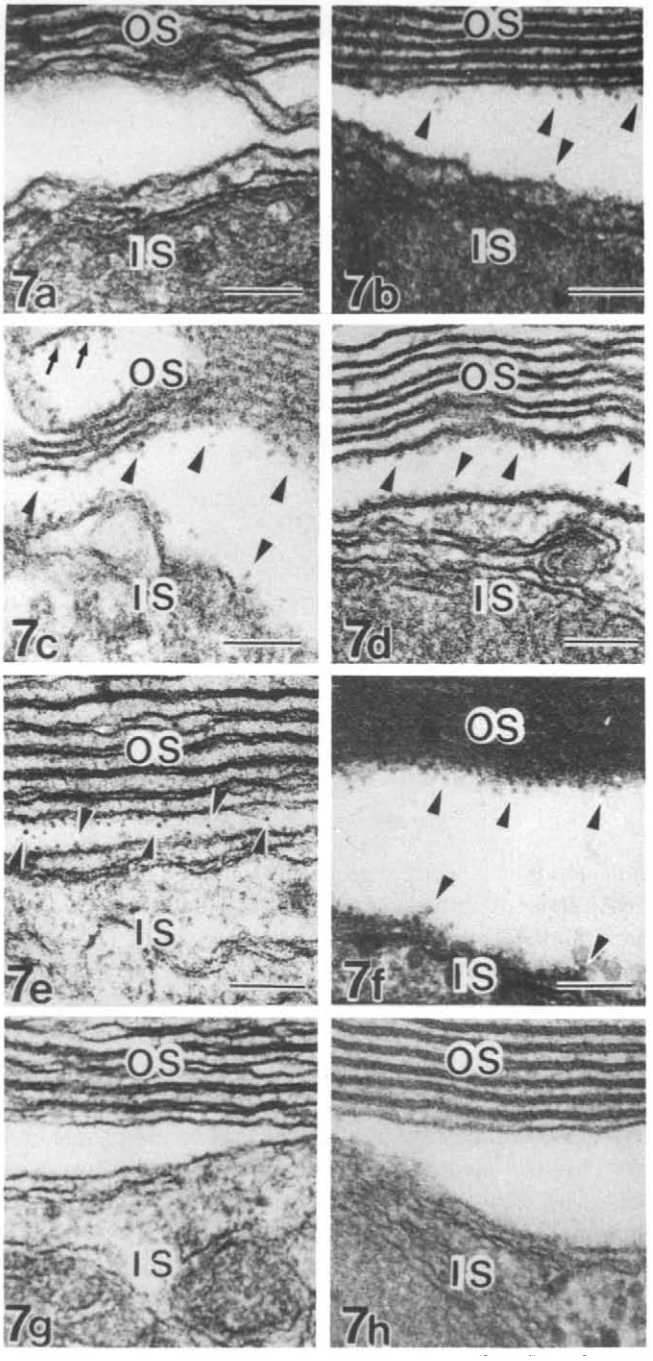

FIGURE 7. Proximal surface of the outer segment (OS) and opposing distal surface of the inner segment (IS) of long (a) and short cell (b) after incubation with Fer-GS. In the long cell, there seem to be no ferritin particles on each surface. Note a number of ferritin particles (arrowheads) on the proximal surface of the outer segment of short cell, in contrast to the scarcity of particles on the opposing distal surface of the inner segment. Opposing surfaces of the outer and inner segments of long (c) and short cell (d) after incubation with Fer-Con A. There are less numerous ferritin particles (arrowheads) on the proximal surface of the outer segment of long cell than those on the corresponding surface of short cell. Note a number of ferritin particles on the luminal surface of the outer segment of long cell (arrows). Distal surfaces of the inner segments of both cells were labeled with Fer-Con A. Opposing surfaces of the outer and inner segments of long (e) and short cell (f) after incubation with Fer-WGA. Note almost the same number of Fer-WGA particles (arrowheads) on the proximal surface of the outer segment of long and short cell. A number of ferritin particles are also present on the distal surface of the inner segment of both long and short cells. Opposing surfaces of the outer and inner segments of long (g) and short cell (h) after incubation with Fer-UEA. Each membrane surface shows negative in UEA labeling. Bars = 0.1 μm, $\times$90,000.

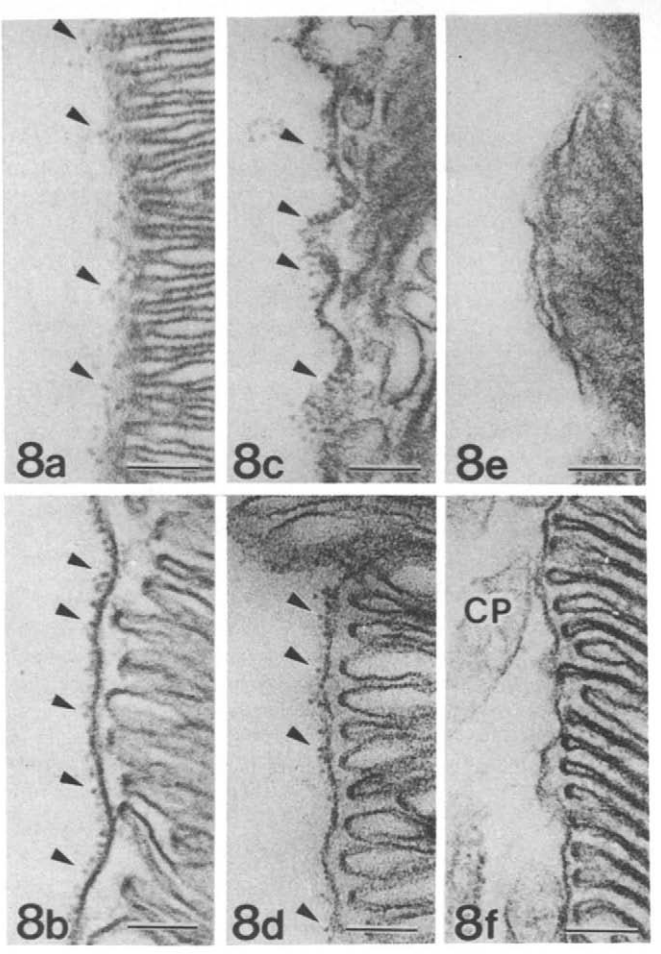

FIGURE 8. Lateral surfaces of the outer segments of long (a) and short cell (b) after incubation with Fer-Con A. Note ferritin particles (arrowheads) on the lateral surface of the outer segment of long cell, and more numerous on that of short cell. Lateral surfaces of the long (c) and short cell outer segments (d) after incubation with Fer-WGA. Note more numerous Fer-WGA particles (arrowheads) on the lateral surface of the outer segment of long cell. Lateral surfaces of the outer segments of long (e) and short cell (f) after incubation with Fer-UEA. Note the negative labeling of the lateral surface of the outer segments of both cells. CP, calycal process; bars = 0.1 μm, $\times$90,000.

difference in the labeling density on the short cell outer segment between Con A and WGA. Lectin binding to its receptor has been known to be enhanced or inhibited by the presence of the adjacent carbohydrate chains (Wu, 1984). Therefore, it is suggested that WGA binding to rhodopsin might be reduced by steric hindrance, especially on the lateral surface of the short cell outer segment.

Fer-WGA has been known to be bound to the rod—but not cone—associated IPM in the monkey retina (Sameshima *et al.*, 1987; Fariss *et al.*, 1990). However, the WGA-positive IPM has not been found around the short cell and Fer-WGA directly binds to the disk or plasma membranes of the long cell outer segments. The corresponding result is obtained in the study of PNA-positive IPM, which cannot be found around the long cell. It has been considered that the retinal IPM is a major route by which nutrients and metabolites pass between photoreceptor cells and the pigment epithelium (Johnson *et al.*, 1986). It seems difficult to determine whether the

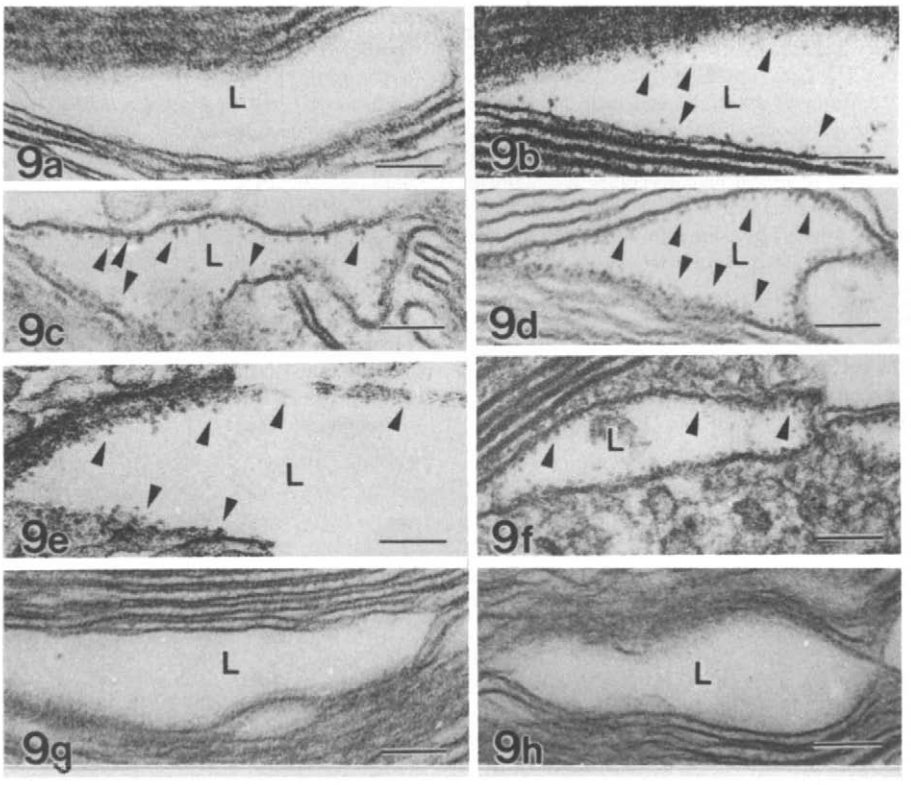

FIGURE 9. Freeze-thawed membranous disks of the long (a) and short cell outer segments (b) after incubation with Fer-GS. Note a number of ferritin particles (arrowheads) confined to short cell disks, in contrast to the absence in long cell disks. L, luminal cavity. Freeze-thawed membranous disks of the long (c) and short cell outer segments (d) after incubation with Fer-Con A. Note a number of Fer-Con A particles (arrowheads) in long cell disks, and more numerous in short cell disks. Freeze-thawed membranous disks of the long (e) and short cell outer segments (f) after incubation with Fer-WGA. Note almost the same number of ferritin particles (arrowheads) in long and short cell disks. Freeze-thawed membranous disks of the long (g) and short cell outer segments (h) after incubation with Fer-UEA. There seem to be no ferritin particles on the luminal surfaces of the outer segments in both cells. Bars = 0.1 μm, ×90,000.

present results represent species differences in the presence of PNA-binding IPM, or the loss of IPM during specimen preparation. In the former case, the absence of WGA- or PNA-positive IPM might indicate that lamprey photoreceptors are nourished in a different manner to higher vertebrates with the IPM.

REFERENCES

Bishop, P. N., Boulton, M., Mcleod, D. & Stoddart, R. W. (1993). Glycan localization within the human interphotoreceptor matrix and photoreceptor inner and outer segments. *Glycobiology*, *3*, 403–412.

Blanks, J. C., Hageman, G. S., Johnson, L. V. & Spee, C. (1988). Ultrastructural visualization of primate cone photoreceptor matrix sheaths. *Journal of Comparative Neurology*, *270*, 288–300.

Blanks, J. C. & Johnson, L. V. (1983). Selective lectin binding of the developing mouse retina. *Journal of Comparative Neurology*, *221*, 31–41.

Blanks, J. C. & Johnson, L. V. (1984). Specific binding of peanut lectin to a class of retinal photoreceptor cells. A species comparison. *Investigative Ophthalmology and Visual Science*, *25*, 546–557.

Bridges, C. D. B. & Fong, S.-L. (1980). Lectins as probes of glycoprotein and glycolipid oligosaccharides in rods and cones. *Neurochemistry*, *1*, 255–267.

Fariss, R. N., Anderson, D. H. & Fisher, S. K. (1990). Comparison of photoreceptor-specific matrix domains in the cat and monkey retinas. *Experimental Eye Research*, *51*, 473–485.

Fliesler, S. J. & Basinger, S. F. (1985). Tunicamycin blocks the incorporation of opsin into retinal rod outer segment membranes. *Proceedings of the National Academy of Science USA*, *82*, 1116–1120.

Fukuda, M. N., Papermaster, D. S. & Hargrave, P. A. (1979). Rhodopsin carbohydrate. Structure of small oligosaccharides attached at two sites near the NH_2 terminus. *Journal of Biology and Chemistry*, *254*, 8201–8207.

Hageman, G. S. & Johnson, L. V. (1986). Biochemical characterization of the major peanut-agglutinin-binding glycoproteins in vertebrate retinae. *Journal of Comparative Neurology*, *249*, 499–510.

Hicks, D. & Molday, R. S. (1985). Localization of lectin receptors on bovine photoreceptor cells using dextran-gold markers. *Investigative Ophthalmology and Visual Science*, *26*, 1002–1013.

Ishikawa, M., Watanabe, H., Koike, Y., Hisatomi, O., Tokunaga, F. & Tonosaki, A. (1989). Demonstrated by lectin cytochemistry of rod and cone photoreceptors in the lamprey retina. *Cell Tissue Research*, *256*, 227–232.

Johnson, L. V., Hageman, G. S. & Blanks, J. C. (1986). Interphotoreceptor matrix domains ensheath vertebrate cone photoreceptor cells. *Investigative Ophthalmology and Visual Science*, 27, 129–135.

Kivela, T. & Tarkkanen, A. (1987). A lectin cytochemical study of glycoconjugates in the human retina. *Cell Tissue Research*, *249*, 277–288.

Lis, H., Sela, B.-A., Sachs, L. & Sharon, N. (1970). Specific inhibition by *N*-acetyl-D-galactosamine of the interaction between soybean agglutinin and animal cell surfaces. *Biochemica et Biophysica Acta*, *211*, 582–585.

Lotan, R., Skutelsky, E., Danon, D. & Sharon, N. (1975). The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *Journal of Biology and Chemistry*, *250*, 8518–8523.

Lyer, P. N., Wilkinson, K. D. & Goldstein, I. J. (1976). An *N*-acetyl-D-

glucosamine binding lectin from *Bandeiraea simplicifolia* seeds. *Archives of Biochemistry and Biophysics*, *177*, 330–333.

Nicolson, G. L., Blanstein, J. & Etzler, M. E. (1974). Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma. *Biochemistry*, *13*, 196–204.

Nir, I. & Hall, M. O. (1979). Ultrastructural localization of lectin binding sites on the surface of retinal photoreceptors and pigment epithelium. *Experimental Eye Research*, *29*, 181–194.

O'Brien, P. J. (1976). Rhodopsin as a glycoprotein: A possible role for the oligosaccharide in phagocytosis. *Experimental Eye Research*, *23*, 127–137.

Olive, J. (1980). The structural organization of mammalian retinal disk membrane. *International Review of Cytology*, *64*, 107–169.

Röhlich, P. (1976). Photoreceptor membrane carbohydrate on the intradiscal surfaces of retinal rod disks. *Nature*, *263*, 789–791.

Sameshima, M., Uehara, F. & Ohba, N. (1987). Specialization of the interphotoreceptor matrices around cone and rod photoreceptor cells in the monkey retina, as revealed by lectin cytochemistry. *Experimental Eye Research*, *45*, 845–863.

Sharon, N. & Lis, H. (1972). Lectins: Cell-agglutinating and sugar-specific proteins. *Science*, *177*, 949–959.

Söderström, K.-O. (1988). Lectin binding to the human retina. *Anatomy Records*, *220*, 219–223.

Steinemann, A. & Stryer, L. (1973). Accessibility of the carbohydrate moiety of rhodopsin. *Biochemistry*, *12*, 1499–1502.

Uehara, F., Sameshima, M., Muramatsu, T. & Ohba, N. (1983). Localization of fluorescence-labeled lectin binding sites on photoreceptor cells of the monkey retina. *Experimental Eye Research*, *36*, 113–123.

Wu, A. M. (1984). Differential binding characteristics and applications of DGalβ1→3DGalNAc specific lectins. *Molecular Cell Biochemistry*, *61*, 131–141.